SUPPORTING INFORMATION

Mucosal Effects of Tenofovir 1% Gel

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Legends for supplementary figures

Figure S1. Correlation of $\log_2$ fold gene induction by tenofovir 1% gel from baseline to Day VII between 9 and 15 cm biopsies. All 137 genes at 9 cm significantly induced ($FDR < 0.05$) and with $\log_2$ fold change > 0.5 are included. Genes depicted as blue dots were significantly induced in both 9 and 15 cm biopsies (15 cm $FDR < 0.05$), genes depicted as black dots were only significant in 9 cm biopsies (15 cm $FDR \geq 0.05$).

Figure S2. Log_2 fold gene suppression (A) and induction (B) from baseline to Day VII caused by N-9 and tenofovir in 9 cm biopsies. (A) All genes with an estimated $FDR \leq 0.05$ and a $\log_2$ fold expression change of ≤ 0.5 are included (N-9: 56 genes; tenofovir: 505 genes). **(B)** All genes with an estimated $FDR \leq 0.05$ and a $\log_2$ fold expression change of ≥ 0.5 are included (N-9: 60 genes; tenofovir: 137 genes). Differences in magnitude of gene expression changes were statistically compared between N-9 and tenofovir by unpaired Mann-Whitney tests. The box plots indicate medians and interquartile ranges and the whiskers indicate 10th to 90th percentiles.

Figure S3. Immunohistochemistry for CD7, and immunofluorescence for CD3, in rectal biopsies before (0) and after seven days (VII) of daily tenofovir 1% gel use. Three representative study participants each are shown and indicated by letters. Blue indicates nuclei, red indicates CD3 or CD7.

Figure S4. Immunohistochemistry for IL-10 and ubiquitin D (UBD) in rectal biopsies before (0) and after seven days (VII) of daily tenofovir 1% gel use. Three representative

study participants each are shown and indicated by letters. Blue indicates nuclei, brown indicates IL-10 or UBD.

Figure S5. Average strength of gene induction by tenofovir 1% gel across all 8 microarray study participants.

Heat map colors depict fold-change at Day I and Day VII over baseline. Baseline is depicted as the vertical bar labeled "0" filled with the shade of blue corresponding to a fold-change of 1. Genes included in the list exhibited ≥ 1.6 fold average induction on Day VII or were ≥ 1.1 fold induced in at least six of eight study participants, and some knowledge about the gene products exists in the literature. The heat map divisions signify three different kinetic patterns of gene induction: flat at day I and then induced; induced at day I and then flat; or induced at day I and then more strongly induced at day VII.

Figure S6. Selected biological processes defined in the InnateDB database with significant enrichment of genes suppressed or induced by tenofovir 1% gel at Day VII in 9 cm biopsies. Green bars depict the percentage of genes identified as suppressed in a particular process out of the total number of genes included by InnateDB in that process. Red bars depict corresponding percentages for gene induction. Numbers of suppressed and induced genes are indicated above the bars. Gene enrichment in each biological process was tested for statistical significance as described in the Methods and the computed p values are depicted by the stars. Not all processes with significant gene enrichment are shown.

Figure S7. Unbiased mass spectrometry proteomics in rectal secretions from five study participants each in the tenofovir and the no treatment arm. (A) 483 proteins were consistently detected in all ten study participants. Of these, 382 proteins in the tenofovir arm had $\log_2$ values > 0 for fold change between baseline and after seven daily gel applications, and

112 had $\log_2$ fold change values > 0 in the no treatment arm. Log_2 fold changes (x axis) and p values signifying the likelihood of change (y axis) for these proteins are shown. Upper panel, tenofovir arm (382 proteins); lower panel, no treatment arm (112 proteins). Data for proteins with $\log_2$ fold values ≤ 0 were not interpretable and are not shown. **(B)** Log_2 fold changes of the top 100 proteins upregulated between baseline and after seven days of daily gel application in each of the five study participants in the tenofovir arm. **(C)** Selected biofunctional processes defined in the Ingenuity database with significant enrichment of proteins induced in rectal secretions by seven days of daily tenofovir 1% gel use. The red bars with arrow heads depict the z scores for these biofunctions, indicating the strength of the directionality of the effect. Protein enrichment in each biofunction was tested for statistical significance as described in the Methods and the computed p values are depicted by the stars. Numbers of induced proteins are indicated above/below the bars.

Figure S1

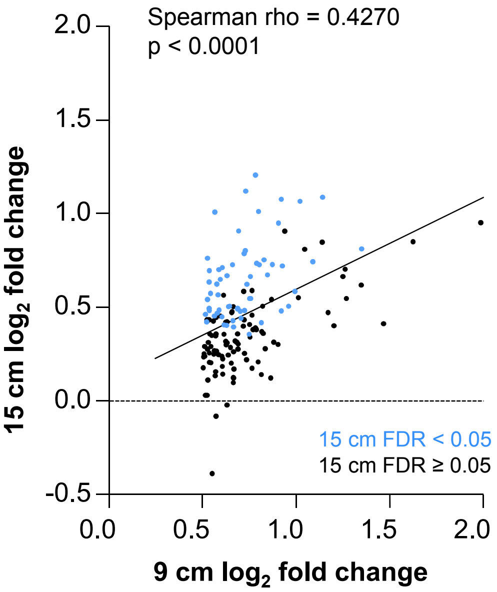

Spearman rho = 0.4270
p < 0.0001
15 cm log2 fold change
9 cm log2 fold change
2.0
1.5
1.0
0.5
0.0
-0.5
0.0
0.5
1.0
1.5
2.0
15 cm FDR < 0.05
15 cm FDR ≥ 0.05

Figure S2

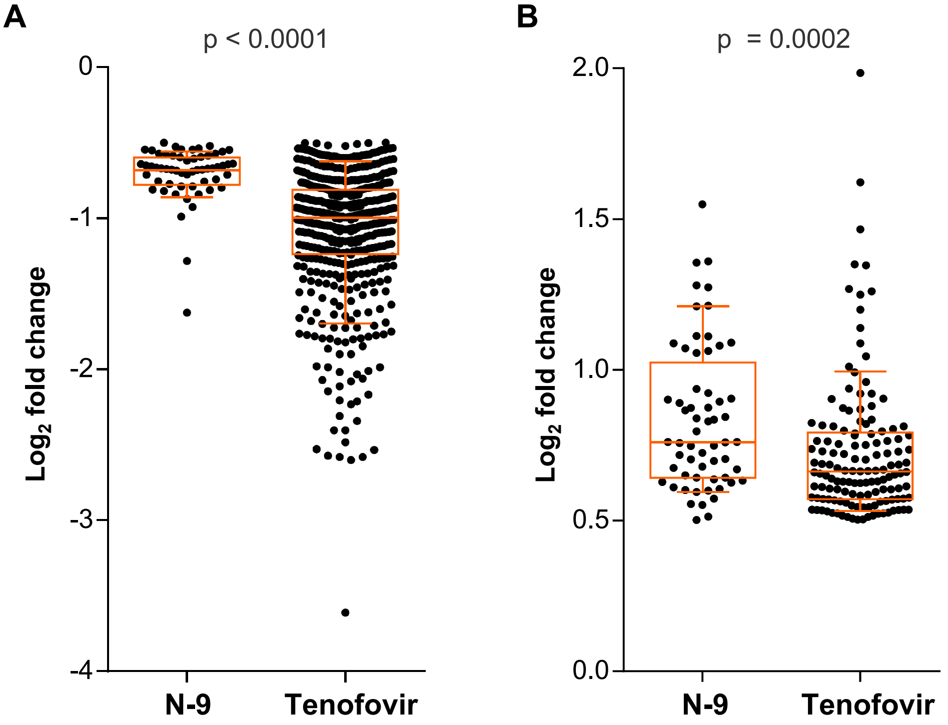

A
p < 0.0001
0
-1
-2
-3
-4
Log2 fold change
N-9
Tenofovir
B
p = 0.0002
2.0
1.5
1.0
0.5
0.0
Log2 fold change
N-9
Tenofovir

Figure S3

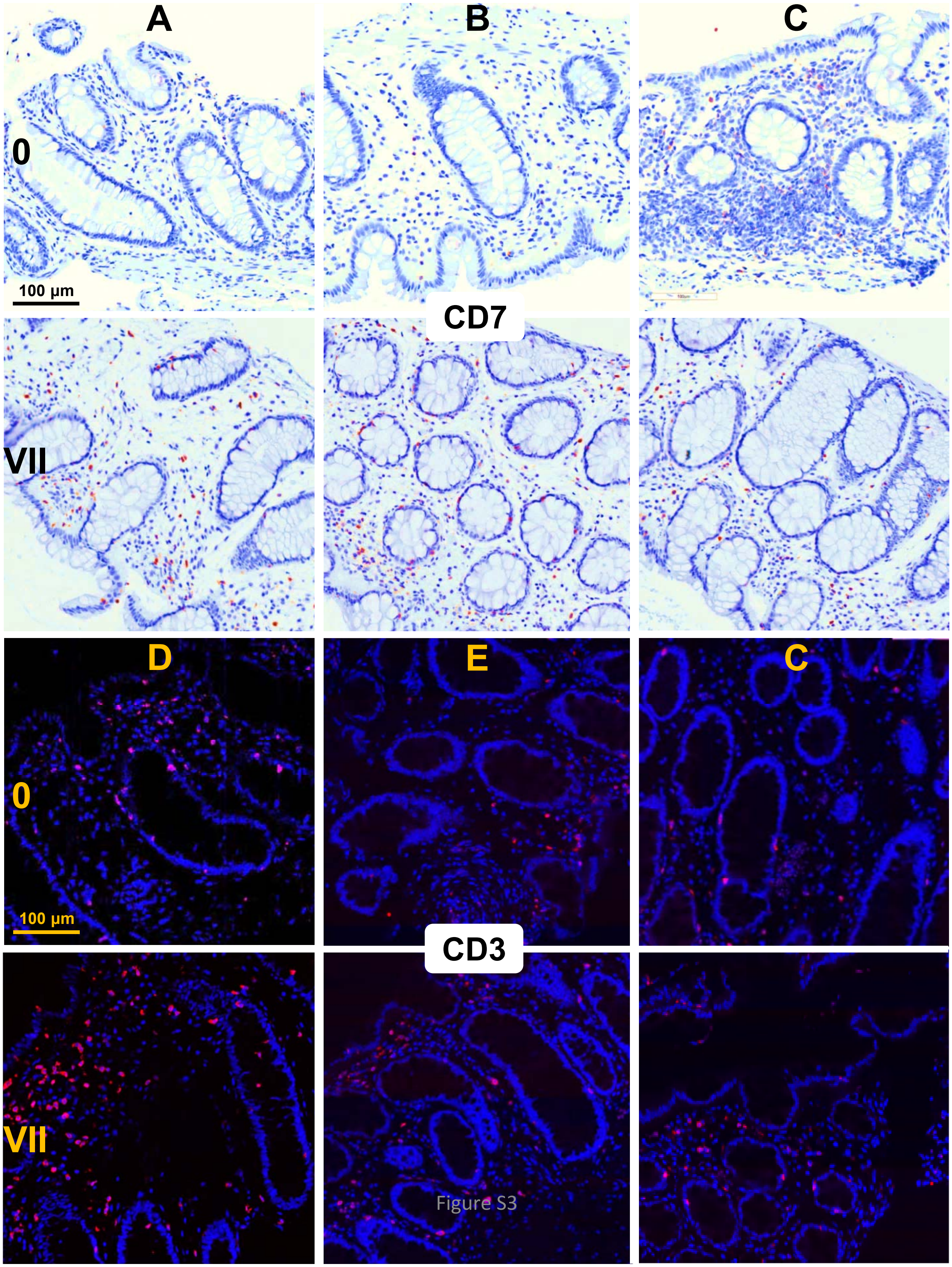


Figure S4

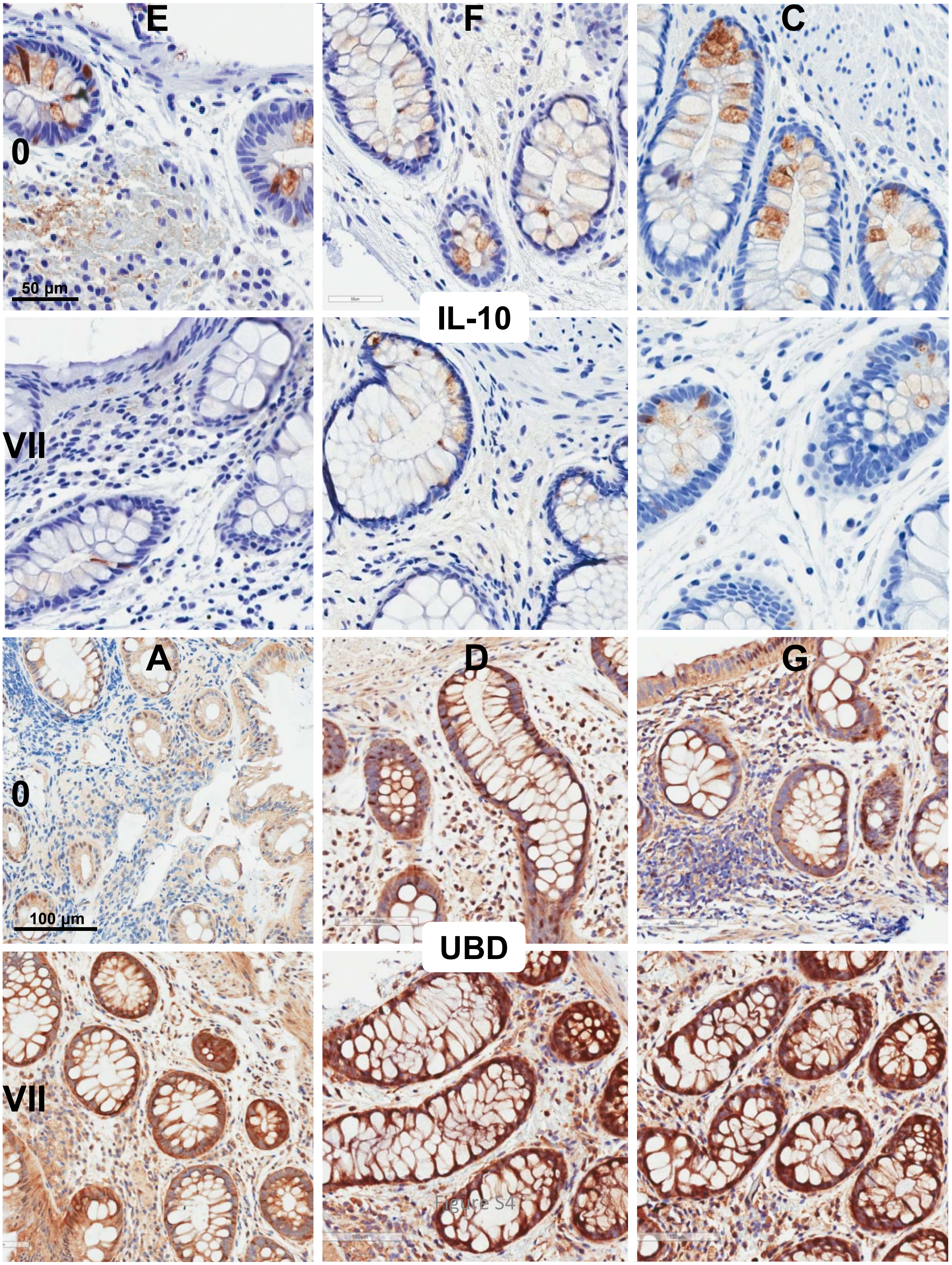


Figure S5

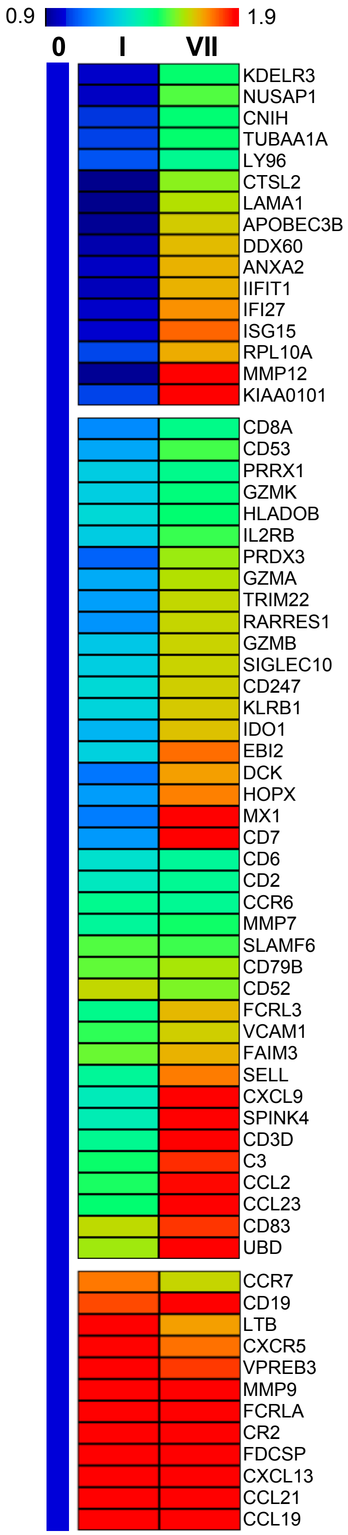


Figure S5

Figure S6

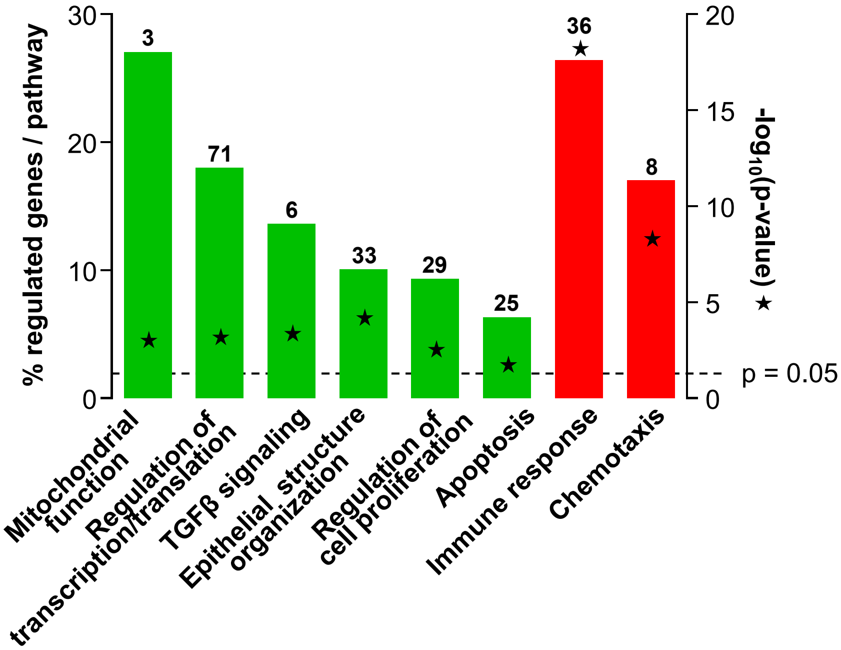

% regulated genes / pathway
30
20
10
0
3
71
6
33
29
25
36
8
★
20
15
10
5
0
-log$_{10}$(p-value) ★
p = 0.05
Mitochondrial function
Regulation of transcription/translation
TGFβ signaling
Epithelial structure organization
Regulation of cell proliferation
Apoptosis
Immune response
Chemotaxis

Figure S7

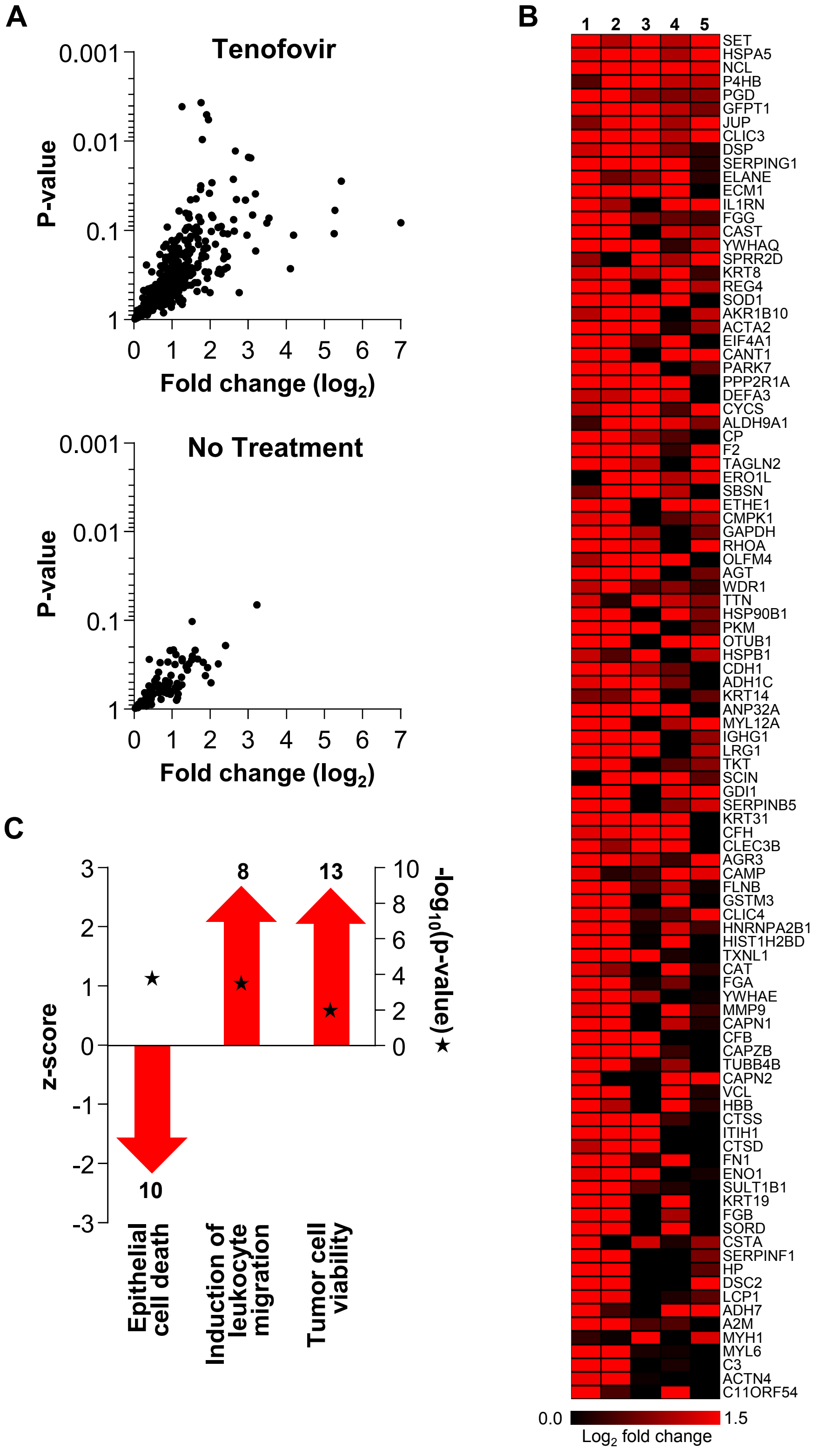
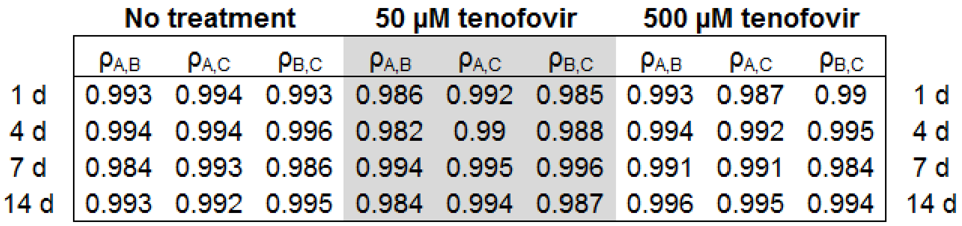

A
Tenofovir
0.001
0.01
0.1
1
P-value
0
1
2
3
4
5
6
7
Fold change ($\log_2$)
No Treatment
0.001
0.01
0.1
1
P-value
0
1
2
3
4
5
6
7
Fold change ($\log_2$)
B
1
2
3
4
5
SET
HSPA5
NCL
P4HB
PGD
GFPT1
JUP
CLIC3
DSP
SERPING1
ELANE
ECM1
IL1RN
FGG
CAST
YWHAQ
SPRR2D
KRT8
REG4
SOD1
AKR1B10
ACTA2
EIF4A1
CANT1
PARK7
PPP2R1A
DEFA3
CYCS
ALDH9A1
CP
F2
TAGLN2
ERO1L
SBSN
ETHE1
CMPK1
GAPDH
RHOA
OLFM4
AGT
WDR1
TTN
HSP90B1
PKM
OTUB1
HSPB1
CDH1
ADH1C
KRT14
ANP32A
MYL12A
IGHG1
LRG1
TKT
SCIN
GDI1
SERPINB5
KRT31
CFH
CLEC3B
AGR3
CAMP
FLNB
GSTM3
CLIC4
HNRNPA2B1
HIST1H2BD
TXNL1
CAT
FGA
YWHAE
MMP9
CAPN1
CFB
CAPZB
TUBB4B
CAPN2
VCL
HBB
CTSS
ITIH1
CTSD
FN1
ENO1
SULT1B1
KRT19
FGB
SORD
CSTA
SERPINF1
HP
DSC2
LCP1
ADH7
A2M
MYH1
MYL6
C3
ACTN4
C11ORF54
0.0
1.5
Log_2 fold change
C
3
2
1
0
-1
-2
-3
z-score
10
8
6
4
2
0
$-\log_{10}$(p-value) ★
★
★
★
8
13
10
Epithelial cell death
Induction of leukocyte migration
Tumor cell viability

Table S1. Pearson correlation coefficients of pre-processed microarray probe expression values between the three primary vaginal cell cultures.



	No treatment			50 µM tenofovir			500 µM tenofovir			
	$\rho_{A,B}$	$\rho_{A,C}$	$\rho_{B,C}$	$\rho_{A,B}$	$\rho_{A,C}$	$\rho_{B,C}$	$\rho_{A,B}$	$\rho_{A,C}$	$\rho_{B,C}$	
1 d	0.993	0.994	0.993	0.986	0.992	0.985	0.993	0.987	0.99	1 d
4 d	0.994	0.994	0.996	0.982	0.99	0.988	0.994	0.992	0.995	4 d
7 d	0.984	0.993	0.986	0.994	0.995	0.996	0.991	0.991	0.984	7 d
14 d	0.993	0.992	0.995	0.984	0.994	0.987	0.996	0.995	0.994	14 d

A, B and C signify the three primary vaginal epithelial cell cultures
d, days of treatment